Ram Semen Processing, Cryopreservation and Cervical Insemination Protocol

Semen Processing

The concentration and motility of the semen sample are determined using spectrophotometry and a Hamilton Thorne motility analyzer (Beverly, MA), respectively (at least 5 fields of analysis and 500 cells). Semen samples are diluted to 1200 x 10^6 sperm/mL in 37 °C skim milk-egg yolk ***cooling*** media (see recipe below). The tubes are then placed in a 37 °C water jacket and cooled to 5 °C over 45 minutes.

The samples are then diluted drop-wise over 5 min (1:1; volume to volume) with 5°C skim milk-egg yolk ***freezing*** media (see recipe below) resulting in a final sperm concentration of 600 x 10^6 sperm/mL and finally loaded into 0.5 mL semen straws.

Cryopreservation

Samples can be frozen one of two ways:

1) Box freezing: Samples are placed on a rack and frozen in liquid nitrogen vapor (4 cm above liquid nitrogen) for 10 min and plunged into the liquid nitrogen for storage.
2) Programmable freezer: The samples are frozen using the Cryo Bio System Mini Digitcool UJ400 (IMV Corporation, Minneapolis, MN) with the following curve: 5 °C to -10 °C at 5 °C/min; -10 °C to -130°C at 60 °C/min and then plunged into liquid nitrogen for storage.

Cryopreserved samples are thawed for 30 seconds in a 37°C water bath.

Semen Cryopreservation Media Recipe from : Paulenz et al., 2007

Skim milk-egg yolk ***cooling*** media:

Dilute 11 grams of non-fat dried skim milk into approximately 80 mL of distilled/deionized water and heat this solution to 95 °C for 10 min. Allow the solution to cool to room temperature and then add 5 mL of egg yolk and 1mg/ml streptomycin sulfate. Mix the solution until homogeneous.

Skim milk-egg yolk ***freezing*** media:

This solution is created by adding 7.3 mL of glycerol to 45 mL of the skim milk-egg yolk cooling media and then mixing until the solution is homogeneous.

Both of these solutions can be frozen in aliquots until the day of use when they can be thawed and used as described.

Cervical Artificial Insemination

The estrous cycles of ewes are synchronized using either:

Sponges for 14 days (e.g. Chronogest CR containing 40 mg fluorogestone acetate, Intervet, Milton Keynes, UK) and then given PMSG (400 IU, i.m.; total volume = 4mL from an 18 gauge needle; single injection) 48 hours prior to sponge removal;

-or-

CIDRs (e.g. 0.3 g progesterone in an inert silicone elastomer for 12 days; Pfizer Animal Health, New York, NY) with PMSG (400 IU i.m. as described previously).

The ewes are then inseminated twice at 53 and 57 hours following sponge/CIDR withdrawal with a single 0.5 mL straw (contains a minimum of 100 x 10^6 motile sperm) and a sheep and goat AI gun (e.g. All-2-Mate Goat Gun, Continental Plastics, Delavan, WI). The labium are parted and the insemination gun is inserted upward at a 45° angle. When the gun touches the top of the vagina it is tilted into a horizontal position and gently inserted into the cervix without force. The insemination dose is placed as deep as possible in the cervix of the ewe without cervical manipulation or force. All inseminations are performed with the ewe restrained in a standing position in a sheep handling squeeze chute.

Reference:

Heiko Paulenz, Tormod Ådnøy and Lennart Söderquist . 2007. Comparison of fertility results after vaginal insemination using different thawing procedures and packages for frozen ram semen. Acta Veterinaria Scandinavica. 49:26.

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